

**Comparison of Effects of Different Activation Treatments on Development of Rabbit Embryos Reconstituted with Fetal Fibroblast**

HJ Lee, JG Yoo, SR Cho\*, SL Lee, JR Chong, HJ Yeo, JM Hwang,  
JS Park, EH Yea, GJ Rho, SY Choe  
College of Veterinary Medicine, Division of Applied Life Science\*  
Gyeongsang National University

To produce reconstituted rabbit embryos with fetal fibroblasts, the present study was evaluated the efficiencies of the different fusion and activation conditions as assessments of subsequent development and chromosome in the embryos. New Zealand White rabbits were used throughout the study. Fetal fibroblasts collected from 22-d of fetuses were cultured in DMEM + 10% FBS in 5% CO<sub>2</sub> in air. The culture was maintained for 10 passages. In every passage half of cell suspension were kept in frozen. From rabbits treated with FSH in 30% PVP solution and hCG, oocytes were surgically collected from oviducts at 14 h post-hCG injection and stripped off their cumulus cells by re-pipetting in a 300 IU hyaluronidase solution. Oocytes with an extruded first polar body and dense cytoplasm were enucleated by micromanipulation in Ham's F-10 medium + 7.5 g/ml cytochalasin B. Enucleation was confirmed under a fluorescence microscope after staining with 5 g/ml bisbenzimidazole for 2 min. Each enucleated oocyte was injected with a fetal fibroblast into a perivitelline space. Reconstructed eggs were compared fusion rates either at 2.0 KV/cm or 1.6 KV/cm (60 sec, double pulses). After fusion, all eggs were activated with the combination of 5 M ionomycin (5 min) and 10 g/ml cycloheximide (CHX, 3h), and cultured in CR1aa medium and transferred into TCM199+10% FBS on day 3. Although there was not significantly differ in fusion rate between treatments (60%, 2.0 KV/cm vs. 79.4%, 1.6 KV/cm), none of them in the eggs fused with 2.0 KV/cm developed to blastocyst. In comparison of development and chromosome status between different activation treatments (Group 1; 5 M ionomycin/10 g/ml CHX, Group 2; 5 M ionomycin/5 g/ml CHX + 2 mM DMAP after fusion with 1.6 KV/cm), there were not differ in cleavage and development rates (67.3% and 28.9% in Group 1; 67% and 33% in Group 2). All out of 8 embryos evaluated in Group 1 appeared a normal diploid chromosome sets and mean number of cells (Mean SEM) on day 4.5 of culture was 141.5 23.15 (n= 8). It can be concluded that the use of cycloheximide has not happened in chromosome abnormalities, and fetal fibroblasts can be used for cloning in rabbit.

(Key words: cycloheximide, 6-dimethylaminopurine, reconstituted embryo, fetal fibroblast, rabbit)

Supported by the MAF-SGRP (Ministry of Agriculture and Forestry Special grants research program, 300012-5) in Korea.